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Recent Research on Nickel Carcinogenesis

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Research on nickel carcinogenesis from 1975 to March 1980 is reviewed. Epidemiological studies have strengthened the evidence that workers in nickel refineries have increased risks of cancers of the nasal cavities and lungs. Clinical investigations have resulted in improved diagnosis, classification, and management of cancers of respiratory organs in nickel refinery workers. Carcinogenicity tests have demonstrated the carcinogenicity of nickel subsulfide (α-Ni_3S_2) in rodents following administration by a variety of parenteral routes. Radiotracer studies and x-ray diffractometry have clarified the metabolism of α-Ni_3S_2 in rodents. *In vitro* exposures of mammalian cells to certain nickel compounds have been shown to inhibit cellular uptake of thymidine-^{3}H, and to induce chromosomal aberrations, somatic mutations, and morphological transformation. Mutagenicity tests of nickel compounds in bacterial systems have consistently been negative. Ni(II) has been reported to impair the fidelity of viral and bacterial DNA polymerases for *in vitro* replication of synthetic nucleotide templates.

Introduction

Research on nickel carcinogenesis prior to 1975 has been comprehensively reviewed and critically evaluated by panels of scientific experts under the auspices of the U.S. National Academy of Sciences (NAS) (*1*) and the International Agency for Research on Cancer (IARC) (*2*). Both scientific panels concluded that increased incidences of lung cancer and nasal cancer have been demonstrated by epidemiological studies of nickel refinery workers, and that the carcinogenicity of certain nickel compounds has been definitely established by animal experiments (*1, 2*). In 1977, the U.S. National Institute of Occupational Safety and Health (NIOSH) stated that "An excess number of deaths from lung cancer and nasal cancer has been observed in nickel refinery workers. After review of the relevant data, it was concluded that a substantial portion of those excess deaths was caused by exposure to airborne nickel compounds" (*3*). Readers are referred to the NAS, IARC, and NIOSH monographs (*1-3*) and to several review articles (*4-9*) for the scientific background on nickel carcinogenesis. Relevant investigations from 1975 to March 1980 are summarized in the present article, so that readers may be informed about the many recent developments in nickel carcinogenesis. Emphasis is placed upon new experimental techniques to study nickel carcinogenesis *in vivo* and *in vitro*, and attention is focused upon prospects for future research on the mechanism(s) whereby nickel compounds initiate neoplastic transformation.

Epidemiological Studies

Several recent studies have analyzed the risks of respiratory tract cancers in workmen who have been exposed to inhalation of nickel compounds (*10-15*). Doll et al. (*10*) reinvestigated the causes of death of employees at a nickel refinery in Clydach, Wales, U.K. based upon a cohort of 967 men who began working before 1945. As shown in Table 1, men who entered employment prior to 1930 had excess risks of respiratory tract cancer. Pedersen et al. (*11*) reported a similar updated survey of mortality from respiratory cancers in workers at a nickel refinery in Kristiansand, Norway. Increased risks of cancers of the nasal cavities, lung and larynx occurred in a cohort of 2249 men who

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worked at the refinery for at least 3 years prior to 1953. Twenty cases of nasal cancer were observed (O) versus 0.81 expected (E) (O/E = 24.7); 69 cases of lung cancer were observed versus 18.64 expected (O/E = 3.7), and 6 cases of larynx cancer were observed versus 2.11 expected (O/E = 2.8). Workers in the roasting, smelting, and electrolysis departments of the nickel refinery had the highest incidence rates for cancers of respiratory organs. Kreyberg (*12*) studied the characteristics of lung cancers in workmen at the same nickel refinery in Kristiansand, Norway. A tabulation of 39 lung cancers included 26 epidermoid (squamous cell) carcinomas, 6 small cell anaplastic carcinomas, and 7 adenocarcinomas (including related histological types). Based upon detailed analyses of work chronologies and smoking histories, Kreyberg (*11*) concluded that tobacco smoking contributed to the development of lung cancers in the nickel-exposed workers. Lessard et al. (*13*) studied the influence of nickel exposure upon lung cancer mortality in Noumea, New Caledonia. Workers at a nickel refinery had three-fold risk of lung cancer, independent of the effects of age and cigarette smoking. Lung cancer risk was also increased three-fold in persons who lived in a zone less than 1 km from the nickel refinery, in comparison to persons who lived more than 3 km from the refinery. This excess risk was independent of employment in the refinery. Bernacki et al. (*14*) assessed the possible association between exposure to nickel-containing compounds and lung cancer mortality in workmen at an aircraft engine factory in Hartford, Connecticut, U.S.A. This case-control study was limited to men who died prior to retirement from work, and hence the possible latent period for development of lung cancer was restricted. The 42 nickel-exposed decedents comprised welders, electroplaters, metal-powder sprayers, grinders, polishers, and bench mechanics, and the 84 control decedents comprised workers in other factory trades who had minimal occupational exposures to nickel. The proportions of deaths from lung cancer were equal in the nickel-exposed decedents and in the controls. Godbold and Tompkins (*15*) performed a case-control study of mortality from respiratory cancer in 814 white men who had been employed prior to 1954 in the barrier department of a gaseous diffusion plant in Oak Ridge, Tennessee, U.S.A. The workers used nickel powder to fabricate a porous barrier for isotopic enrichment of uranium by gaseous diffusion. The control group included 1600 white men who worked in other departments and who had no record of nickel exposure. Employees and pensioners were traced for a minimum follow-up period of 19 years. The nickel-exposed cohort experienced lower mortality than the controls, both in deaths from respiratory cancer and in deaths from all causes, but neither of these differences was statistically significant (*15*).

Clinical Investigations

Recent advances in diagnosis, classification, and management of cancers of the nasal cavities and lungs in nickel refinery workers have been described in clinical reports (*16-23*). Nelen et al. (*16*) conducted a prospective study of sputum cytology in 268 asymptomatic men who had been employed prior to 1963 in the sintering department of a nickel refinery in Sudbury, Ontario, Canada. Eleven cases of lung cancer and one case of laryngeal cancer were discovered. Nelen et al. (*16*) concluded that prospective cytologic screening of sputum for neoplastic cells is an effective technique for detection of cancers of the respiratory tract in nickel refinery workers. They also noted that 11 of the 12 subjects with respiratory tract cancers were smokers and that the remaining subject was a former smoker (*16*). Barton (*17*) summarized the results of a cancer

Table 1. Deaths from respiratory tract cancers in a cohort of workmen at a nickel refinery in Clydach, Wales.[a]

	Deaths from cancer of the nasal sinuses[b]			Death from cancer of the lung[b]		
Year of first employment	O[c]	E	O/E	O	E	O/E
< 1910	14	0.036	389	24	2.389	10.0
1910-1914	24	0.037	649	34	3.267	10.4
1915-1919	11	0.025	440	20	3.070	6.5
1920-1924	7(1)	0.071	99	50	9.642	5.2
1925-1929	0(1)	0.026	0	9	3.613	2.5
1930-1944	0	0.034	0	8	5.463	1.5

[a]Data of Doll et al. (*10*).

[b]O = observed deaths based upon death certificates; E = expected deaths based upon national mortality rates; O/E = ratio of observed to expected deaths.

[c]Cases of nasal sinus cancer referred to as an associated cause of death are shown in parentheses.

detection program for approximately 1200 workmen at a nickel refinery in Kristiansand, Norway. The program consisted of periodic physical examinations (including rhinoscopy), x-ray examinations of the chest and nasal sinuses, and analyses of nickel concentrations in plasma and urine. In selected cases, sputum cytology and nasal mucosal biopsy were performed. During 5 years of surveillance, nearly 100 employees with suspicious signs or symptoms were subjected to nasal mucosal biopsies. The biopsies yielded the following significant findings: 4 cases of invasive squamous cell carcinoma, 2 cases of carcinoma *in situ*, and 16 cases of epithelial atypia. Fifteen of these subjects had worked in the roasting-smelting department of the nickel refinery. Barton (*17*) discussed the management of nasal cancers in nickel refinery workers and he recommended radical surgical resection, alone, or in combination with postoperative radiation therapy. Torjussen and co-workers (*18-23*) evaluated the rhinoscopic appearance, x-ray findings, histopathologic lesions, and nickel concentrations in body fluids and nasal biopsies of active and retired workers at the same nickel refinery in Kristiansand, Norway. Nasal polyps and hyperplastic rhinitis were more common in the nickel refinery workers than in controls, but no significant differences were observed between x-ray findings in nasal sinuses of nickel-exposed and control subjects (*18*). Histopathologic changes in nasal biopsies from active and retired nickel refinery workers and from controls were scored numerically, and the scores were found to correlate with duration of nickel exposure, type of nickel exposure, and tobacco consumption. Two workers from the roasting-sintering department, both employed 28 years at the nickel refinery, had nasal carcinomas. Epithelial dysplasia, an apparently precancerous lesion, was found in 38 of 318 active and 7 of 15 retired nickel workers, and in only 1 of 57 controls (*19, 20*).* Increased nickel concentrations were found in nasal biopsy specimens from all categories of nickel-exposed workers, but the highest concentrations were found in workers from the roasting-smelting department (*21*). Analyses of nickel concentrations in nasal biopsy specimens from pensioned workers showed that accumulated nickel was retained for several years after termination of nickel exposure, and was slowly released from the nasal mucosa with an estimated half-life of 3.5 years (*21*). Attempts to identify the cellular localization of nickel in nasal biopsy specimens by histochemical staining methods and by energy dispersive x-ray microanalysis were inconclusive, owing to insufficient analytical sensitivity (*22, 23*). Sunderman (*24*) reported an unusual case of polypoid squamous cell carcinoma of the nose in a 36-year-old man. The patient had worked in a cutlery factory for 12 years. For several years, he had immersed small nickel-plated objects (such as teapots) in a tank of HCl-HNO_3 at 85°C in order to remove old nickel plating (e.g., from battered hotel utensils). During this operation, the patient had chronically inhaled nickel-containing acid fumes from the nickel-stripping tank. In view of the patient's relative youth and occupational history, Sunderman (*24*) suspected that the patient's nasal cancer was caused by nickel. Bourasset and Galland (*25*) previously reported a similar case of nasal cancer in a cutlery worker who had been exposed to inhalation of nickel-containing fumes.

*In view of the propensity of wood-workers to develop nasal cancer, it is noteworthy that the control subject with epithelial dysplasia was a carpenter.

Carcinogenicity Tests in Animals

In order to bring up to date the IARC monograph on nickel and nickel compounds (*2*), this résumé of recent tests of the carcinogenicity of nickel compounds in experimental animals is patterned on the IARC format.

Inhalation and/or Intratracheal Administration

Saknyn and Blohkin (*20*) exposed nonpedigree albino rats to inhalation of feinstein dust (an intermediate product of nickel refining, which contains NiS, NiO, and metallic Ni) in atmospheric concentration of 70 mg dust/m^3 for 5 hr/day, 5 days/week during 6 months. Lung cancers (squamous cell carcinomas) were found in 2 of 5 rats that survived the treatment. The latent period for tumor development was 17 months. Saknyn and Blohkin (*20*) also administered black nickel monoxide (NiO) to albino rats as a single intratracheal injection (20 to 40 mg/rat). Lung cancer (squamous cell carcinoma) developed in 1 of 26 rats after a latent period of 17 months. No lung tumors were found in an untreated control group of 47 rats.

Mukubo (*27*) treated female albino rats by a single intratracheal injection of metallic nickel dust (10 mg/rat), alone, or in combination with methylcholanthrene (5 mg/rat). At 12 weeks, lung cancers (squamous cell carcinomas) were seen in (a) 3 of 5 rats that received nickel plus methylcholanthrene; (b) 2 of 7 rats that received methylcholanthrene alone, and (c) 0 of 7 rats that received nickel alone. The failure to detect lung cancers in the nickel-treated group should not be considered a negative

carcinogenicity test, owing to the short period of observation.

Yarita and Nettesheim (*28*) studied the carcinogenicity of nickel subsulfide (α-Ni_3S_2) in heterotopic tracheas that were transplanted (two tracheas/rat) under the dorsal skin of isogeneic female rats of the Fischer strain. Gelatin pellets were inserted into the transplanted tracheas at 4 weeks after grafting. Sixty tracheas received pellets that contained 1 mg of α-Ni_3S_2; 64 tracheas received pellets that contained 3 mg of α-Ni_3S_2, and 10 control pellets received gelatin pellets, alone. Surviving rats were killed after 20 months. At the 1 mg dose of α-Ni_3S_2, tumors developed in 9/60 tracheas (5 squamous cell carcinomas, 1 undifferentiated carcinoma, 2 fibrosarcomas, and 1 leiomyosarcoma). At the 3 mg dose of α-Ni_3S_2, tumors developed in 45/64 tracheas (1 squamous cell carcinoma, 12 fibrosarcomas, 10 leiomyosarcomas, 10 fibromyosarcomas, 2 rhabdomyosarcomas, 2 fibromyxosarcomas, 7 sarcomas of uncertain type, and 1 benign myoma). No tumors developed in the 10 control tracheas.

Oral Administration

Sunderman et al. (*29*) painted α-Ni_3S_2 in glycerol onto the buccal pouch mucosa of four groups of Syrian golden hamsters of the LVG/LAK strain. The dosage schedules were: (a) 1 mg α-Ni_3S_2, 3 times/week for 18 weeks in 6 hamsters; (b) 2 mg α-Ni_3S_2, 3 times/week for 18 weeks in 7 hamsters; (c) 5 mg α-Ni_3S_2, 3 times/week for 36 weeks in 15 hamsters; and (d) 10 mg α-Ni_3S_2, 3 times/week for 36 weeks in 13 hamsters. Surviving hamsters were killed at 24 months after the initial application. No tumors were found in the buccal pouch, oral cavity or gastrointestinal tract of any of these hamsters, or in 15 controls that received buccal pouch applications of the glycerol vehicle (0.2 ml, 3 times/week for 36 weeks). Cancers (squamous cell carcinomas) of the buccal pouch were found in 4/4 hamsters in a positive control group that received similar applications of dimethylbenzanthracene in glycerol (1 mg DMBA, 3 times/week for 18 weeks).

Intramuscular Injection

Sunderman (*30*) administered α-Ni_3S_2 to albino mice of both sexes by a single IM injection (2.5 mg α-Ni_3S_2/mouse). Within 100 weeks, sarcomas developed at the injection site in 6/9 mice of the DBA-2 strain (versus 0/9 vehicle controls) and in 5/10 mice of the C57-BL6 strain (versus 0/9 vehicle controls).

Sunderman et al. (*31*) gave single IM injections of six nickel compounds in equal doses (14 mg Ni/rat) to male Fischer rats. This experiment was a supplement to an earlier study (*32*) that was included in the 1976 IARC monograph (*2*). By 100 weeks after the IM injection, the incidences of sarcomas at the injection sites were: (a) metallic Ni dust: 13/20 rats; (b) crystalline nickel subselenide (Ni_3Se_2): 21/23 rats; (c) crystalline nickel monoselenide (NiSe): 8/16 rats; (d) crystalline nickel subsulfide (α-Ni_3S_2): 9/9 rats; (e) crystalline nickel monosulfide (β-NiS): 14/14 rats; (f) amorphous nickel monosulfide (NiS): 0/10 rats; and (g) vehicle controls: 0/44 rats. Based upon the marked differences in sarcoma incidences after an IM injection of crystalline β-NiS and amorphous NiS, Sunderman et al. (*31*) concluded that the physical form of nickel sulfides has a critical influence upon their carcinogenic activities. In the same experiment, sarcomas were observed at the injection site in 3/16 rats that received a lower dose (7 mg Ni/rat) of nickel carbonyl-cyclopentadiene dimer; $[Ni(CO)_2(C_5H_5)_2]_2$.

Sunderman (*30*) derived a dose-response curve for induction of sarcomas in male Fischer rats by single IM injection of α-Ni_3S_2, based upon four published and two previously unpublished experiments which involved a total of 383 rats. The experiments were all terminated at 100 to 104 weeks after the injection. Sarcoma incidence at 62 weeks after the injection was linearly related to the reciprocal of the α-Ni_3S_2 dose, and ranged from 24% (7/29) in rats that received 0.63 mg of α-Ni_3S_2, to 100% (9/9) in rats that received 20 mg of α-Ni_3S_2. There was no indication of a threshold carcinogenic dosage in this experimental system. The histologic types of 336 sarcomas induced by an IM injection of α-Ni_3S_2 included 161 rhabdomyosarcomas, 91 undifferentiated sarcomas, 72 fibrosarcomas, 9 liposarcomas, 2 neurofibrosarcomas, and 1 hemangiosarcoma. Metastases were found in 41% (137/336) of tumor-bearing rats.

Sunderman et al. (*33*) gave male Fischer rats a single IM injection of α-Ni_3S_2 (1.2 mg/rat), alone, or in combination with metallic Mn dust (1.0 mg/rat) or metallic Cr dust (1.0 mg/rat). Within 100 weeks, the sarcoma incidence in rats that received only α-Ni_3S_2 was 22/30. Addition of Mn dust to α-Ni_3S_2 reduced the sarcoma incidence to 1/14, whereas addition of Cr dust to α-Ni_3S_2 did not affect the sarcoma incidence (12/15). No sarcomas developed at the IM injection site in three control groups, including 39 rats that received the injection vehicle, 14 rats that received Mn dust alone (1.0 mg/rat), and 15 rats that received Cr dust alone (1.0 mg/rat).

Sunderman (*29*) administered α-Ni_3S_2 to male Syrian golden hamsters of the LVG/LAK strain by a single IM injection. By 24 months after the injection, the incidence of local sarcomas was 5/15 in hamsters that received 5 mg of α-Ni_3S_2 and 12/17 in

hamsters that received 10 mg of α-Ni_3S_2. Metastases were found in 10 sarcoma-bearing hamsters. No sarcomas occurred at the injection site in 14 control hamsters that received an IM injection of the NaCl vehicle.

Hildebrand and Biserte (*34*, *35*) described 16 sarcomas (including unspecified numbers of rhabdomyosarcomas, fibrosarcomas, and 3 leiomyosarcomas) that developed in albino rabbits at the site of IM implantation of α-Ni_3S_2 in agar (80 mg α-Ni_3S_2/rabbit). These papers were concerned primarily with the ultrastructural features of the tumors. The numbers of treated and control rabbits were not specified.

Intraperitoneal Injection

Stoner et al. (*36*) and Shimkin et al. (*37*) described an experiment in which nickelous acetate was administered to 3 groups of strain A mice (20 mice/group) by IP injection, 3 times/week for 8 weeks, for total dosages of 72, 180, and 360 mg/kg, respectively. A control group was given similar IP injections of the vehicle. The mice were killed at 30 weeks after the first injection. The average number of lung tumors/mouse was 0.42 in controls, 0.67 at the 72 mg/kg dose; 0.71 at the 180 mg/kg dose, and 1.26 at the 360 mg/kg dose. At the highest dose level, the increase in lung tumors was statistically significant.

Saknyn and Blokhin (*26*) treated albino rats by single IP injection of feinstein dust at a dosage of 90-150 mg dust/rat. Sarcomas developed at the injection site in 6 of 39 rats after latent periods of 6 to 15 months.

Intrarenal Injection

Jasmin and associates (*38*, *39*) administered α-Ni_3S_2 to female Sprague-Dawley rats by intrarenal (IR) injection in dosage of 10 mg/rat. In three separate experiments, cancer of the injected kidney developed in 7/16, 11/24, and 11/20 rats, respectively, within 12 months. In contrast, renal cancers did not develop in two control groups of 20 and 16 rats which received IR injection of the vehicle, or in two groups of 18 and 20 rats which received IR injection of either metallic Ni dust or NiS (10 mg/rat). The renal tumors in α-Ni_3S_2-treated rats were all classified as carcinomas, although many of the tumors were pleomorphic and included anaplastic spindle-cell varieties. Jasmin and Solymoss (*39*) mentioned unsuccessful attempts to induce renal tumors in mice, hamsters and rabbits by IR injection of α-Ni_3S_2, but they did not furnish experimental details.

Sunderman et al. (*40*) also tested the carcinogenicity of α-Ni_3S_2 following IR injection in rats. Tumors of the injected kidney developed within 2 years in 9/32 Fischer rats (14 female, 18 male) that received 5mg of α-Ni_3S_2, and in 23/38 rats (14 female, 24 male) that received 10 mg of α-Ni_3S_2. No renal tumors occurred in 52 control Fischer rats (17 female, 35 male) that received IR injection of the NaCl vehicle. Injection IR of α-Ni_3S_2 (5 mg/rat) induced renal tumors in 6/12 NIH black rats (6 female, 6 male), and 7/11 Wistar-Lewis rats (6 female, 5 male). In contrast, no renal tumors developed in 12 α-Ni_3S_2-treated Long-Evans rats (6 female, 6 male). In male Fischer rats that received an IR injection of α-Ni_3S_2 (10 mg/rat) combined with metallic Mn dust (7 mg/rat), the incidence of renal tumors was 17/28, which differed significantly from the corresponding incidences of 18/24 and 0/23 in male Fischer rats that received IR injections of α-Ni_3S_2 (10 mg/rat) alone or Mn dust (7 mg/rat) alone. The 54 renal tumors that were observed by Sunderman et al. (*40*) in α-Ni_3S_2-treated rats were all malignant, and metastases were found in 37/54 tumor-bearing rats. The authors were uncertain whether the renal cancers were epithelial or mesenchymal in origin (*40*).

Intratesticular Injection

Damjanov et al. (*41*) administered α-Ni_3S_2 to male Fischer rats by single intratesticular injection (10 mg α-Ni_3S_2/rat). Within 20 months, malignant testicular neoplasms developed in 16 of 19 α-Ni_3S_2-treated rats, and in 0 of 18 controls that received intratesticular injection of NaCl vehicle. The testicular neoplasms in α-Ni_3S_2-treated rats included 4 fibrosarcomas, 4 fibrous histiocytomas, and 4 rhabdomyosarcomas. Metastases were identified in 4/16 tumor-bearing rats.

Intraocular Injection

Albert et al. (*42*) injected α-Ni_3S_2 (0.5 mg/rat) into the vitreous cavity of the right eye of juvenile male Fischer rats (1 month old). Malignant ocular tumors developed in 14 of 15 treated rats by 8 months, and in 0 of 11 controls which received an intraocular injection of NaCl vehicle. In three α-Ni_3S_2-treated rats, the injected eye had two primary tumors, and in two α-Ni_3S_2-treated rats, the injected eye had three distinct primary tumors. The 22 ocular neoplasms included 11 amelanotic uveal melanomas, 4 retinoblastomas, 3 gliomas, 1 fibrosarcoma and 1 phakocarcinoma, and 3 unclassified malignant tumors. Extraocular extension, invasion of the optic nerve, and metastases to lung and brain were noted.

Intracerebral Injection

Sosinski (*43*) injected nickelic oxide (Ni_2O_3) into the cerebral cortex of 20 Wistar rats (10 male, 10 female) in a dosage of 3 mg Ni_2O_3/rat. Each rat also received an im injection of Ni_2O_3 (10 mg/rat) into the left gastrocnemius muscle. Control rats were not mentioned. Cerebral gliomas were observed in two rats that were killed at 14 and 21 months, respectively, and a meningioma was found in one rat that was killed at 21 months. No neoplasms developed at the sites of IM injection of Ni_2O_3.

Other Parenteral Routes

Jasmin and Solymoss (*39*) mentioned that IV administration of α-Ni_3S_2 (10 mg/rat) to 20 female Sprague-Dawley rats did not increase the incidence of benign or malignant tumors, and that intrahepatic administration of α-Ni_3S_2 (10 mg/rat) to eight female Sprague-Dawley rats did not induce any hepatic tumors. The periods of observation were not specified. Sunderman et al. (*29*) reported that no hepatic tumors developed in 13 male Fischer rats that received an intrahepatic injection of α-Ni_3S_2 (5 mg/rat), nor did any salivary gland tumors develop in 11 male Fischer rats that received an injection of α-Ni_3S_2 (2.5 mg/rat) into a submaxillary gland. The periods of observation were 2 years (*29*).

Relevant Experiments in Animals

X-Ray Diffractometry and Radiotracer Studies

The metabolism of α-Ni_3S_2 in rodents has been investigated by x-ray diffractometry (*44*, *45*) and by radiotracer studies (*33*, *44-46*). Applications of nickel radioisotopes in biological research have recently been comprehensively reviewed by Kasprzak and Sunderman (*47*). These authors emphasized that ^{63}Ni is an ideal radioisotope for investigations of metal carcinogenesis, because ^{63}Ni is available in high specific activity (up to 3.87 kCi/g-atom) and has a long half-life (92 years). Moreover, the soft beta emission of ^{63}Ni (67 keV) is readily counted by liquid scintillation spectrophotometry, and it provides autoradiograms with exceptionally high resolution. Kasprzak (*46*) administered α-Ni_3S_2 that was radiolabeled with ^{63}Ni or ^{35}S to Fischer rats by IM injection in both hind limbs (10 mg/injection). Local sarcomas developed in 8/8 α-$^{63}Ni_3S_2$-treated rats and in 4/7 α-$Ni_3{}^{35}S_2$-treated rats during 8 months of observation. Tumors developed at one injection site in 5/15 rats, and at both injection sites in 7/15 rats. The tumors were all pleomorphic rhabdomyosarcomas. Autoradiography showed extracellular particles of α$^{63}Ni_3S_2$ and α$Ni_3{}^{35}S_2$ at the injection sites. The particles of radiolabeled α-Ni_3S_2 eventually became surrounded by neoplastic tissue. Intracellular localization of ^{63}Ni or ^{35}S was not detected within muscle or tumor cells, but sparse α-$^{63}Ni_3S_2$ particles were seen within macrophages at the injection sites (*46*).

Sunderman et al. (*33*) elucidated the metabolism of ^{63}Ni following single IM injection of α-$^{63}Ni_3S_2$ in 10 male Fischer rats (1.2 mg/rat). The cumulative excretions of ^{63}Ni during 8 weeks after the injection averaged 67 (S.D. ± 2) % in urine and 7 ± 2 % in feces. At 2 to 10 weeks after IM injection of α-Ni_3S_2, the particles of α-Ni_3S_2 which remained at the injection site were predominantly intracellular, and were located primarily within cytoplasmic vesicles in fibroblasts and macrophages. Residual ^{63}Ni at the injection site averaged 19 ± 4% of the dose in rats killed at 20 to 24 weeks, and 14 ± 2% of the dose in rats killed at 31 weeks after the injection. Whole-body ^{63}Ni kinetic parameters which were computed by compartmental analysis were not affected by admixture of Mn dust based upon measurements of ^{63}Ni in urine, feces, injection site, and viscera of rats that received an IM injection of α-$^{63}Ni_3S_2$ (1.2 mg/rat), alone, or in combination with Mn dust (1.0 mg/rat). However, the subcellular distribution of ^{63}Ni derived from α-$^{63}Ni_3S_2$ was significantly changed by admixture of Mn dust (*33*). ^{63}Ni concentrations in ultrafiltrates of supernatant fractions of homogenates of injection sites averaged 2.8 ± 0.7 ng/ml at 20 to 24 weeks after injection of α-$^{63}Ni_3S_2$ plus Mn dust, versus 5.4 ± 2.0 ng/ml after injection of only α-$^{63}Ni_3S_2$.

Oskarsson et al. (*45*) administered α-$^{63}Ni_3S_2$ or α-$Ni_3{}^{35}S_2$ to NMRI mice by an IM or SC injection (10 mg/mouse). During the period from 2 to 14 months after the injection, local sarcomas developed in 11/16 mice that received a SC injection of α-Ni_3S_2 labelled with ^{63}Ni or ^{35}S, and in 8/16 mice that received a similar IM injection of α-Ni_3S_2. In the tumors, radiolabelled particles of α-Ni_3S_2 were mostly intracellular within fibroblasts and macrophages. X-ray diffractometry of the insoluble residue from lyophillized tumor tissue did not reveal α-Ni_3S_2, but distinctly demonstrated crystalline α-Ni_7S_6 and β-NiS. Whole-body autoradiography showed gradual mobilization of solubilized ^{63}Ni and ^{35}S from the injection site. There was also mobilization of nonsolubilized ^{63}Ni-labeled particles, which were located within phagocytes in liver, spleen, and regional lymph nodes (*45*). These *in vivo* findings are compatible with earlier observations of Kasprzak

and Sunderman (*44*), who employed x-ray diffractometry to elucidate the dissolution of α-Ni_3S_2 during *in vitro* incubation in rat serum. α-Ni_3S_2 was slowly oxidized to crystalline nickel monosulfide (β-NiS), which subsequently underwent further oxidation to yield soluble Ni(II) complexes and relatively insoluble particles of nickel hydroxide [$Ni(OH)_2$] (*44*).

Electron Microscopic Studies

Bruni (*48*) administered α-Ni_3S_2 to male Sprague-Dawley rats by unilateral or bilateral IM injection into the thigh muscles (20 mg/injection). The rats were sacrificed at intervals from 2 to 26 weeks, and tissue from the injection site was examined by electron microscopy. Mitotic activity was seen primarily in muscle satellite cells. Satellite cells in division were morphologically indistinguishable from dividing stem cells in α-Ni_3S_2-induced rhabdomyosarcomas. On the basis of these findings, Bruni (*48*) suggested that muscle satellite cells are progenitors of the α-Ni_3S_2-induced tumors.

Hildebrand and Biserte (*49*) performed electron microscopy of 12 rhabdomyosarcomas that were induced in an unspecified number of Wistar rats by IM implantation of α-Ni_3S_2 in agar (20 mg α-Ni_3S_2/rat). Successive stages of differentiation of tumor cells were described, and formation of microtubules in interphase rhabdomyoblasts was convincingly demonstrated. Hildebrand and Biserte (*49*) did not observe any satellite cells in the rhabdomyosarcomas. In another study, Hildebrand and Biserte (*35*) described cylindrical paracrystalline structures in rhabdomyoblasts of rhabdomyosarcomas that were induced in rabbits by IM injection of α-Ni_3S_2 in agar (80 mg α-Ni_3S_2/rabbit). The authors speculated that the laminated cylindrical bodies represented abnormal aggregates of contractile proteins that were synthesized during myofibrillar differentiation.

Jasmin et al. (*50*) treated 25 female Sprague-Dawley rats by IR injection of α-Ni_3S_2 in glycerol (5 mg α-Ni_3S_2/rat). Groups of five rats were killed at biweekly intervals until 2 months, and the injected kidneys were examined by electron microscopy. Unusual crystalline inclusions were observed in mitochondria of tubular cells that were located in the pars recta of the distal nephron. By goniometric analysis, the authors deduced that the crystalline inclusions were composed of cylindrical rods in a hexagonal array. Jasmin et al. (*50*) speculated that the crystalline inclusions might consist of abnormal assemblages of protein components of mitochondrial cristae.

Cytogenetic and Tumor Transplantation Studies

Yamashiro et al. (*51*) performed karyotypic analyses and transplantation experiments on rhabdomyosarcomas that were induced in Fischer and Long-Evans rats by single IM injection of α-Ni_3S_2 (10 mg/rat). The chromosomal complements of tumor cells from 12 primary rhabdomyosarcomas were usually in the diploid range, although 11 of the 12 tumors contained a few triploid and tetraploid cells. Abnormal chromosomes were found, including dicentric, triradial, and ring forms. Comparisons of chromosomes of primary and metastatic tumor cells suggested that tumors with diploid or near-diploid chromosomes were most likely to metastasize. Rhabdomyosarcoma cells were cultured *in vivo* in diffusion chambers which were implanted in the peritoneal cavity of syngeneic rats. Such cell cultures exhibited less myogenic differentiation than parallel cell cultures which were incubated *in vitro* in Leighton tubes (*51*).

Abandowitz (*52*) added neuraminidase to cultured cells from an α-Ni_3S_2-induced rat fibrosarcoma. The neuraminidase treatment inhibited tumor growth following inoculation of the fibroblasts into normal recipient rats. The recipient rats acquired enhanced resistance to subsequent inoculations of tumor cells.

Effects on DNA Synthesis *in Vivo*

Hui and Sunderman (*53*) measured *in vivo* incorporation of thymidine-^3H into DNA in rats at 28 hr after partial hepatectomy. Administration of nickel carbonyl [$Ni(CO)_4$] at 2 or 4 hr before sacrifice inhibited thymidine-^3H uptake into liver and kidney DNA. For example, in rats killed 4 hr after IV injection of $NI(CO)_4$ (2 mg Ni/100 g), ^3H-labeling of liver DNA averaged 54 (SE ± 10) % of controls, and ^3H-labeling of kidney DNA averaged 53 ± 6 % of controls. Injection of $NiCl_2$ (2 mg Ni/100 g, im) 4 hr before sacrifice did not significantly affect thymidine-^3H uptake into liver DNA, but did inhibit thymidine-^3H uptake into kidney DNA (65 ± 6% of controls). Binding of ^{63}Ni to DNA in liver and kidney of rats killed 4 hr after injection of $^{63}Ni(CO)_4$ or $^{63}NiCl_2$ ranged from 0.3 to 2.2 mole ^{63}Ni/mole of DNA nucleotides. The binding of ^{63}Ni to DNA that was observed by Hui and Sunderman (*53*) was consistent with previous reports by Heath and Webb (*54*) and Webb et al. (*55*) of nickel binding to nucleoli, chromatin, and deoxyribonucleohistones isolated from nickel-induced rhabdomyosarcomas. However, the presence of ^{63}Ni in the DNA prepara-

tions did not necessarily connote *in vivo* ^{63}Ni-binding, since the possibility of ^{63}Ni-binding to DNA during tissue homogenization and DNA isolation could not be excluded (*53*). Hui and Sunderman performed ultracentrifugal fractionations of liver DNA on alkaline sucrose gradients (*53*), and they did not observe any differences between sedimentation profiles of liver DNA from $Ni(CO)_4$-treated rats versus paired control rats.

Relevant Studies in Cell Cultures

In vitro exposure of mammalian cells to certain nickel compounds inhibits cellular uptake of thymidine-^{3}H and induces chromosomal aberrations, somatic mutations, and morphological transformation. In studies which were briefly mentioned in the NAS and IARC monographs (*1*, *2*), Basrur and Gilman (*56*) and Swierenga and Basrur (*57*) showed that addition of α-Ni_3S_2 to cultures of rat embryo muscle cells profoundly inhibited thymidine-^{3}H uptake, suppressed cell division, and induced bizarre mitoses, including multipolar and distorted bipolar spindles, C-metaphase-like shapes, and lagging chromosomes. Mitotic arrest occurred in telophase and post-telophase, consistent with disturbed dissolution of mitotic spindles. In a recent study, Nishimura and Umeda (*58*) found that addition of nickel compounds, ($NiCl_2$, NiS, nickel acetate, and potassium cyanonickelate), to cultures of mouse mammary carcinoma cells inhibited thymidine-^{3}H uptake and increased the frequency of chromosomal aberrations. Anacher and Paillet (*59*) reported that exposure of mouse lymphoma cells to $NiCl_2$ caused dose-dependent increases in trifluorothymidine-resistant mutants, and Hsie et al. (*60*) noted that exposure of Chinese hamster ovary cells to $NiCl_2$ induced thioguanine-resistant mutants. Casto et al. (*61*), DiPaolo and Casto (*62*), Pienta et al. (*63*), Costa et al. (*64-66*), and Rivedal and Sanner (*67*) showed that *in vitro* exposures of Syrian hamster embryo cells to $NiSO_4$ or α-Ni_3S_2 resulted in morphological transformation. Casto et al. (*61*) failed to detect DNA damage by alkaline sucrose gradient ultracentrifugation of DNA from cultured hamster embryo cells that had been exposed *in vitro* to $NiSO_4$. Costa et al. (*66*) demonstrated that several clones of α-Ni_3S_2-transformed cells produced fibrosarcomas following SC injection in nude mice. DiPaolo and Casto (*62*) and Costa et al. (*66*) observed dose-dependent relationships between the concentration of α-Ni_3S_2 in the tissue culture medium and the incidence of morphological transformation of Syrian hamster fetal cells. Amorphous nickel monosulfide (NiS) did not induce morphological transformation under the same conditions (*62*, *66*). Costa et al. (*67*) compared *in vitro* uptake of α-Ni_3S_2 and amorphous NiS by Chinese hamster ovary cells and Syrian hamster fetal cells. Both types of cells avidly engulfed α-Ni_3S_2 particles, whereas they only engulfed a few amorphous NiS particles under similar exposure conditions. Costa et al. (*67*) suggested that the striking disparity in carcinogenic activity of crystalline α-Ni_3S_2 and amorphous NiS may be attributed to marked differences in cellular uptake of the two compounds.

Rivedal and Sanner (*68*) employed *in vitro* morphological transformation and induction of somatic mutation to investigate synergism between nickel and polycyclic aromatic hydrocarbons. The transformation frequency of Syrian hamster embryo cells increased with increasing concentrations of $NiSO_4$, benzo(a)pyrene (BP), or methylcholanthrene (MC). When cells were exposed to combinations of $NiSO_4$ and BP, the transformation frequencies were much higher than when the compounds were tested separately. The greatest enhancement was found with 5 μg/ml of $NiSO_4 \cdot 6H_2O$ and 0.78 μg/ml of BP. The transformation frequency obtained with this combination was 10.7%, compared to frequencies of 0.5% and 0.6% that were obtained with the individual substances. No synergistic effect was detected between $NiSO_4$ and MC. In experiments that measured somatic mutations in Syrian hamster embryo cells by selection for ouabain-resistance, the mutation frequency was significantly higher than expected when the cells were exposed to mixtures of $NiSO_4$ and BP (*68*). Rivedal and Sanner's observation of mutagenic synergism between $NiSO_4$ and BP is consistent with an earlier report by Maenza et al. (*69*) of carcinogenic synergism between α-Ni_3S_2 and BP following IM injection in Fischer rats. Sunderman (*70*) observed that exposure of rats to $Ni(CO)_4$ by inhalation or IV injection inhibited phenothiazine induction of BP (arylhydrocarbon) hydroxylase activity in lung and liver. Sunderman and Roszel (*71*) administered BP to rats by IV injection and studied the effect of $Ni(CO)_4$ on the retention of BP in lung and liver. A single exposure of rats to $Ni(CO)_4$ inhibited BP mobilization from lung and liver for 48 hr (*70*). The inhibitory effects of nickel compounds on BP metabolism are of especial interest in view of the potentiating effect of cigarette smoking on the development of lung cancer in nickel refinery workers (*12*, *13*, *16*).

Mutagenicity Tests in Bacteria

Bacterial mutagenesis tests of nickel compounds have consistently been negative, despite several attempts by experienced workers to demonstrate

mutagenicity in *E. coli* or *S. typhimurium* (*7, 72-75*).*

Studies in Biochemical Systems

Sirover and Loeb (*77*) demonstrated that Ni(II), Co(II), and Mn(II) substituted for Mg(II) as activators of avian myeloblastosis virus (AMV) DNA polymerase for replication of synthetic polynucleotide templates. During DNA synthesis by AMV DNA polymerase in the presence of Mg(II), addition of Ni(II) (as well as soluble salts of other carcinogenic metals) decreased the fidelity of DNA replication (*77, 78*). Sirover and Loeb (*78*) suggested that impaired fidelity of DNA replication by AMV DNA polymerase might serve as an *in vitro* screening test to identify metal compounds that could potentially be carcinogenic and/or mutagenic. Miyaki et al. (*79*) found that Ni(II) increased misincorporation of deoxynucleotides by *E. coli* DNA polymerase I during transcription of synthetic polynucleotide templates. Loeb et al. (*80*) and Zakour et al. (*81*) speculated that carcinogenic metals may diminish the fidelity of DNA polymerase activity in target cells *in vivo*, and may thereby induce errors in selection of nucleotide bases during DNA synthesis. According to this hypothesis, decreased fidelity of DNA polymerase might initiate a cascade of random somatic mutations and evolve transformed cells that possess selective advantages for proliferation in the host. This hypothesis and related theories about possible molecular mechanisms of metal carcinogenesis have been considered in recent review articles (*7, 75, 81, 82*).

Prospects for Future Research

The demonstration by Sirover and Loeb (*80*) of metal-induced infidelity of DNA replication may possibly point to a fundamental mechanism of metal carcinogenesis. However, even if this is not the case, their research has attracted the attention of molecular biologists to the previously neglected area of metal carcinogenesis. Recent refinements of techniques to investigate derangements of nucleic acid synthesis, repair, and regulation in eukaryotic cells will undoubtedly facilitate mechanistic studies of metal carcinogenesis. α-Ni_3S_2 is an exceptionally advantageous compound for use in such studies, since α-Ni_3S_2 is inexpensively available in high purity and is readily labelled with ^{63}Ni, a beta-emitting radioisotope with long half-life that is well suited for liquid scintillation counting and autoradiography. The carcinogenic activity of α-Ni_3S_2 is apparently greater than any other metallic compound which has been investigated (*6*). A remarkable variety of animal species, routes of administration and cell culture systems can be employed for cancer research with α-Ni_3S_2. Furthermore, neoplastic transformation by α-Ni_3S_2 can be suppressed by manganese dust *in vivo* and *in vitro* (*30, 33, 40, 64*). This observation may serve as a clue to identify the biochemical effects of α-Ni_3S_2 that are specifically associated with neoplastic transformation.

*Dr. Samuel J. Rogers (Montana State College, Bozeman, MT) did not observe mutations of *S. typhimurium* in the Ames mutagenesis test system with α-Ni_3S_2, NiS, Ni_3S_4, NiTe, NiSb, NiO, and $NiSO_4$, (personal communications on December 3, 1976 and January 26, 1977). Dr. Goran Lofroth (University of California, Berkeley, CA) also obtained negative results in the Ames mutagenesis test system with α-Ni_3S_2, (personal communication on March 3, 1977).

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